Synthesis of Silver Nanoparticles Using the Selected Fruit Peels and Testing their Antibacterial Effectiveness against Urinary Tract Infection Caused By *Escherichia coli*

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INDEX

Sr.No.	CONTENT	Page No.
1.	INTRODUCTION	1-7
2.	REVIEW OF LITERATURE	7-12
3.	RESEARCH OBJECTIVES	13
4.	METHODOLOGY	14-15
5.	SIGNIFICANCE OF THE RESEARCH WORK	16
6.	REFERENCES	17-21

INTRODUCTION

Truly revolutionary nanotechnology products, materials and applications, such as nanorobotics, are years in the future (some say only a few years; some say many years). What qualifies as "nanotechnology" today is basic research and development that is happening in laboratories all over the world. "Nanotechnology" products that are on the market today are mostly gradually improved products (using evolutionary nanotechnology) where some form of nanotechnology enabled material (such as carbon nanotubes, nanocomposite structures or nanoparticles of a particular substance) or nanotechnology process (e.g. nanopatterning or quantum dots for medical imaging) is used in the manufacturing process. In their ongoing quest to improve existing products by creating smaller components and better performance materials, all at a lower cost, the number of companies that will manufacture "nanoproducts" (by this definition) will grow very fast and soon make up the majority of all companies across many industries. Evolutionary nanotechnology should therefore be viewed as a process that gradually will affect most companies and industries.

Nanotechnology and its concepts

Nanotechnology, now a days is booming tremendously in each and every field of science and technology, it has created its special place in the field of medical, chemical and physical sciences, as it has now started to revolutionizing the drug delivery sciences (Jain *et al.., 2011).* Nanotechnology deals with structures sized between 1to100 nanometer in at least one dimension and involve developing materials or devices within that size. Nanometer to a meter is the same as that of a marble to the size of the earth (Kahn, 2006).Nanoparticles possess unique electrical, optical as well as biological properties and thus are used in biossensing, catalysis, imaging, drug delivery, nanodevice fabrication and in medicine (Nair and Laurencin, 2007).Silver nanoparticles have gained interest in the past few years due to their antimicrobial properties (Choi *et al., 2008).*they are even being projected as future generation antimicrobial agents (Rai *et al., 2009).*

There are two main approaches used in nanotechnology “bottom-up” approach” in this approach, materials and devices are built from molecular components which assemble themselves chemically by principles of molecular recognition.

In “top-down” approach nano-objects are constructed from larger control (Rodgers, 2006).

History of Nanotechnology

Professor Norio Taniguchi of Tokyo Science University has defined the term “nanotechnology” in a 1974 paper (Taniguchi, 1974) as follows: “Nano-technology mainly consists of the processing of separation, consolidation, and deformation of materials by one atom or by one molecule.”

In the 1980s the basic idea of this definition was explored in much more depth by Dr. K. Eric Drexler, who promoted the technological significance of nano-scale phenomena and devices through speeches and the books Engines of Creation: The Coming Era of Nanotechnology (1986) and Nanosystems: Molecular Machinery, Manufacturing, and computation (Drexler, 1991), and so the term acquired its current sense.

Nanotechnology and nanoscience got started in the early 1980s with two major developments; the birth of cluster science and the invention of the scanning tunneling microscope (STM). In another development, the synthesis and properties of semiconductor nanocrystals was studied; this led to a fast increasing number of metal and metal oxide nanoparticles and quantum dots.

Federal nanotechnology research and development and is evaluated by the president’s council of advisors on science and technology.

Principle of biosynthesis of Nanoparticles

The production of metal-based nanoparticles by chemical reduction (Peterson *et al.,* 2007), thermal treatment (Sun and Luo, 2005), irradiation (Shao and Yao, 2006) and laser ablation (Tsuji *et al.,* 2002) often times requires the use of organic solvents and toxic reducing agents like sodium borohydride and N,Ndimethylformamide. Therefore, biological and biomimetic approaches for the synthesis of nanomaterials are being explored. Cell mass or extracellular components from microorganisms, such as *Klebsiella pneumoniae, Bacillus licheniformis, Fusarium oxysporum, Aspergillus flavus, Cladosporium cladosporioides, Aspergillus clavatus, and Penicillium brevicompactum* (Ahmad *et al.,* 2003; Shahverdi *et al.,* 2007; Kalishwaralal *et al.,* 2008; Balaji *et al.,* 2009; Shaligram *et al.,* 2009; Verma *et al.,* 2010) have been utilized for the reduction of silver ions to AgNPs. The unexploited plant resources for the synthesis of silver nanoparticles, various plant leaf extracts such as *Helianthus annus, Basella alba, Oryza sativa, Saccharum officinarum, Sorghum bicolour and Zea mays*; *Capsicum annuum L.; Pelargonium graveolens; Carica papaya; Chenopodium album; Rosa rugosa; Jatropha curcas; Aloe vera; boswellia ovalifoliolata* (Leela and Vivekanandan 2008; Shankar *et al.,* 2008; Mude *et al.,* 2008; Dwivedi and Gopal 2010; Dubey *et al.,* 2010; Bar *et al.,* 2009; Chandran *et al.,* 2008; Ankanna *et al.,* 2010).

Development of reliable and eco-friendly process for synthesis of metallic nanoparticles is an important step in the field of application of nanotechnology. One of the options to achieve this objective is to use natural processes such as use of biological systems. One approach that shows immense potential is based on the biosynthesis of nanoparticles using biological waste plant products such as fruit peel. The principle of preparation of silver nanoparticles by using microorganism is a bioreduction process; the silver ions are reduced by the extracellular reductase enzymes produced by the microorganisms to silver metal in nanometer range

Urinary tract infection

Urinary tract infection is one of the most common infection, an adult mostly women experience in their lifetime. Although it does not sound so severe but it creates life, threatening conditions if left untreated like the infections of the bladder and kidneys accompanied by fever, vomiting, tiredness and abdominal pain, which can further lead to kidney damage. Those who suffer from renal stones and other renal problem are more prone to urinary tract infection, as the obstruction caused by the stones in the ureter becomes the reason for the bacterial accumulation in the urinary tract and this becomes the cause of urinary tract infection. (Dulawa J.2004)

Urinary tract infection is a bacterial infection caused by any of the following bacteria:-

Escherichia coli
Klebsiella
Pseudomonas
Staphylococcus
Group D.*Streptococci*
Anaerobes particularly bacteroids

The main causative pathogen involved is Gram negative *E.coli* (Ronald A.2003 the etiology of urinary tract infection. Traditional and emerging pathogen)
Although anyone can get UTI, but women are more prone to this infection than males children of school going age were also easily get encountered by UTI, usually due to unhygienic toilet habits and use of dirty toilets. Whereas, in males the main cause for the bacteria to encounter are prostate enlargement and post surgery.

A German bacteriologist Theodor Escherich first discovered *E.coli* in 1885 inside human colon. The name *Escherichia coli* w-as given to this bacterium to give honor to its discoverer. Most strains of *E.coli* are harmless while some makes you sick with disease like diarrhea and urinary tract infection.

Consequences of urinary tract infection
Strictures, abscess formation, fistulas, bacteraenmia, sepsis, pyelonephritis and kidney dysfunction are all the consequences caused by urinary tract infection bacteria.
Depending upon the severity of the infection it is divided into two categories
Lower urinary tract infection (infection of urethra)
Upper urinary tract infection (infection above the bladder)

Lower UTIs includes the following symptoms

- Urethritis (inflammation of the urethra)
- Cloudy urine
- Dysuria
- Pyuria
- Frequency and urgency to urinate
- Pain
- Rarely blood in urine

Upper UTIs include the following symptoms

- Kidney dysfunction
- Pyelonephritis (inflammation of the parenchyma of the kidney)
- Fever/chills

All these accompanied by the symptoms of lower UTI.
Therefore proper treatment, diagnosis and preventions are necessary to control the infection. UTI have the tendency to reoccur after the primary infection, it is important to bring out the causative bacteria and to treat it properly because if it is left untreated than it shows life threatening outcomes. (Dulawa J 2004).

The Following measurers can do the prevention and cure of the urinary tract infection:-

ANTIBIOTICS

Continuous antibiotics are currently used as treatments for UTIs. The literature recommends treating anywhere from 6 to 12 months to 2 to 5 years (Zak, 2014). Antibiotics are the most popular method being practiced by the physicians and urologist, as they quickly responds to the bacteria. The commonly used antibiotics are trimethoprim-sulfamethoxzole combination antibiotics, beta lactam including penicillin and cephalosporin, amino glycosides example gentamycin, amikacin and tobramycin, but many organisms have resistance to some of these drugs. Nitrofurantoin is occasionally used to treat mild UTIs. Antibiotic to be used is very dependent on the pathological report i.e. culture and sensitivity of the patient's sample.
When antibiotics are used for urinary tract infection useful bacteria are killed along with the harmful bacteria, thus it degrades the immune system of the body, and it becomes the reason for the development of secondary yeast infection and digestive problems.

Antibiotic revival using silver Nanoparticles

Antibiotic resistance is one of the global problems the world is facing today. Bacteria are becoming more powerful by developing resistance against the antibiotics used to combat them. Pharmaceutical industries are also facing great stress due to the drug resistance, as it is the major challenge for them to develop new antibiotics for the resistant bacteria. Therefore, with the help of nanotechnology we will try to revive certain resistant antibiotics effectiveness using silver nanoparticles. Silver nanoparticles synthesized using nanotechnology shows effective antibacterial properties. (Roy S. and Das K.T. 2015) so, we will use the biosynthesized silver nanoparticles in combination with the resistant or least effective antibiotics and will examine the effectiveness by measuring the zone of inhibition against the targeted microbe i.e. *Escherichia coli*.

Characteristics of plant taken

There are many reports present on biosynthesis of silver nanoparticles from plant extract, as per the literature survey, but only few reports are there on the biosynthesis of silver nanoparticles using the waste products of the plant.

In this study, we will take outer hard peel of fruits of three different plants:-

Litchi chinensis, Mangifera indica and Juglans regia.

Litchi chinensis (Litchi)

Classification	
Kingdom	**Plantae**
Order	**Sapindales**
Family	**Sapindaceae**
Genus	***Litchi***
Species	***chinensis***

Delicious and juicy lychee or *"Litchi"*, botanically, this exotic fruit belongs to the family of *Sapindaceae*, and named scientifically as ***Litchi chinensis***.

Litchi has sweet taste, and fragrant flavor. Its flesh envelopes around a single, glossy, brown seed, 2 cm long, and 1–1.5 cm in diameter. The seeds, as that in the case of sapodilla, are not poisonous but should not be eaten. Fresh lychees can be readily available in the markets from May to October, about 120-140 days after flowering. Its outer skin is rough leathery rind or peel, featuring pink color. Its peel can be detached easily in the ripe berries. Inside, its flesh consists of edible portion or aril that is white, translucent, sweet, and juicy. Litchi is cultivated in many parts of the world and used in many forms like in making jam, jellies, juices and candies. Litchi fruit contains 66 calories per 100g; it contains non saturated fats or cholesterol and composes of good amount of vitamins, dietary fiber, and antioxidants. It is an excellent source of vitamin c and minerals like potassium and copper. Therefore, it offers protection against coronary heart diseases.

Nutritional value of litchi

Fresh whole lychee contains a total 72 mg of vitamin C per 100 grams of fruit, an amount representing 119% of the Daily Value .On average, consuming nine peeled lychee fruits would meet an adult's daily vitamin C requirement. Consumption of fruits rich in vitamin C helps human body develop resistance against infectious agents and scavenge harmful, pro-inflammatory free radicals.

1. Mangifera indica (Mango)

Classification	
kingdom	**Plantae**
Order	**Sapindales**
Family	**Anacardiaceae**
Genus	***Mangifera***
Species	***indica***

Mango, the king of all fruits; it is one of the most delicious seasonal fruit grown in tropics. Botanically this fruit belongs within the family of *Anacardiaceae.* The center of diversity of the *Mangifera* genus is in India. Mango trees grow up to 35–40 m (115–131 ft) tall, with a crown radius of 10 m (33 ft).

Each fruit measures 5 to 15 cm in length and 4 to 10 cm in width. Over 400 varieties of mangoes are known, many of which ripen in summer, while some give double crop. The ripe fruit varies in size and color. The fruit takes three to six months to ripen. Its outer skin is green when unripe and turns golden yellow, crimson red to orange. Mango fruit is an excellent source of vitamin-A, vitamin- B6, vitamin- C. Additionally, mango peel is also rich in phytonutrients, such as the triterpene, lupeol.

Phytochemical and nutrient content appears to vary across mango cultivars. Up to 25 different carotenoids have been isolated from mango pulp, the densest of which was beta-carotene, which accounts for the yellow-orange pigmentation of most mango cultivars. Mango leaves also have significant polyphenol content, including xanthonoids, mangiferin and gallic acid.

Nutritional value of mango is as follows:-

Mango fruit is an excellent source of Vitamin-A and flavonoids like beta-carotene, alpha-carotene, and beta-cryptoxanthin. 100 g of fresh fruit provides 765 IU or 25% of recommended daily levels of vitamin-A. Together; these compounds have been known to have antioxidant properties and are essential for vision. Vitamin A is also required for maintaining healthy mucos and skin. Consumption of natural fruits rich in carotenes is known to protect from lung and oral cavity cancers. Additionally, mango peel is also rich in phytonutrients, such as the pigment antioxidants like carotenoids and polyphenols. Mango fruit is rich in pre-biotic dietary fiber, vitamins, minerals, and poly-phenolic flavonoid antioxidant compounds.

2. *Juglans regia (Walnut)*

Classification	
kingdom	**Plantae**
Order	**Fagales**
Family	**Juglandaceae**
Genus	***Juglans***
Species	***regia***

Walnuts are well known for their nutritional values when consumed whole with skin, as 90% of the phenols of walnut are found in its skin.(Fukuda *et al.* 2003) The form of vitamin E found in walnuts is somewhat unusual, and particularly beneficial. Instead of having most of its vitamin E present in the alpha-tocopherol form, walnuts provide an unusually high level of vitamin E in the form of gamma-tocopherol. Particularly in studies on the cardiovascular health of men, this gamma-tocopherol form of vitamin E has been found to provide significant protection from heart problems.
Walnuts are a delicious way to add extra nutrition, flavor and crunch to a meal. While walnuts are harvested in December, they are available year round and a great source of those all-important omega-3 fatty acids. Delicious walnut comes from an ornamental tree that is highly prized for its beauty. The walnut kernel consists of two bumpy lobes that look like abstract butterflies. The lobes are off white in color and covered by a thin, light brown skin. They are partially attached to each other. The kernels are enclosed in round or oblong shells that are brown in color and very hard.

Selected pathogenic microbes

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Escherichia coli

E. coli bacteria were discovered in the human colon in 1885 by German bacteriologist Theodor Escherich. Although *E. coli* bacteria were initially called *Bacterium coli,* the name was later changed to *Escherichia coli* to honor its discoverer. (Eisenstein *et al.*2000). *Escherichia coli (*or *E. coli*) are the most prevalent infecting organism in the family of gram-negative bacteria known as *Enterobacteriaceae. E. coli* is often referred to as the best or most-studied free-living organism. (James M. Jay, 2000) More than 700 serotypes of *E. coli* have been identified. . (Griffin *et al.,* 1991).The "O" and "H" antigens on the bacteria and their flagella distinguish the different serotypes. It is important to remember that most kinds of *E. coli* bacteria do not cause disease in humans. (Feng, Peter, *et al.,* 2002) Indeed, some *E. coli* are beneficial, while some cause infections other than gastrointestinal infections, such as urinary tract infections. The best-known and most notorious *E. coli* bacteria that produce Shiga toxin is *E. coli* O157:H7.

The main microbe involved in the infection of urinary tract is *Escherichia coli* therefore our study emphasize on the control of this bacteria.
Samples from different sources are labeled as mentioned below

1. Escherichia coli 1
2. Escherichia coli 2
3. Escherichia coli 3
4. Escherichia coli 4
5. Escherichia coli 5

Need of the study

Antibiotics, although proved to be life saving, but they create several problems by the continuous usage such as they bring imbalance in the natural protective flora of gastrointestinal tract and urinary tract, thus weakens immune system.
Now a days, pathogens are becoming resistant towards the antibiotics commonly used for the treatment which is becoming the major cause of hindrance in the treatment of even minute infections causing pharmaceutical industry to fear, that how will they develop new antibiotics. Thus, nanotechnology has given us a way out for such a critical condition of multi drug resistance by giving us the concept of nanoparticles, which are synthesized for many beneficial purposes such as for the bacterial treatments as the nanoparticles synthesized by the biogenic process, shows antibacterial properties against many pathogenic bacteria. Therefore using nanotechnology, we will synthesize such nanoparticles, which will show effective antibacterial properties against the bacteria causing urinary tract infection and this study will help in finding a way out of the critical problem of multi drug resistance. Apart nanoparticles are proving beneficial against many life-threaten diseases like cancer, therefore we took a step closer to know and discover more about the essence of nanoparticles. This study helps me in bringing out an alternative for the misuse of antibiotics against disease like urinary tract infection which can be treated in much simpler way rather than using heavy doses of antibiotics which can be kept safe for the other critical illness at critical stage of illness.

REVIEW OF LITERATURE

Nanotechnology is a blessing in the field of science and technology as it has created numerous such technology that is proving beneficial and promising for the sustainable development of the future. Using concept of nanotechnology synthesis of nanoparticles came in account by both chemical and biological process. Developing an environment friendly process for synthesis of nanoparticles is a significant step in the field of nanotechnology. Among the all metallic nanoparticles silver nanoparticles draw more attention due to its unique physical, chemical and biological properties. Green protocol of synthesizing nanoparticles has emerged as an alternative to overcome the limitation of conventional methods. Plant and microorganisms are majorly applied for green synthesis of metallic nanoparticles. Using plants towards synthesis of nanoparticles are emerging and also beneficial compared to microbes with the presence of broad variability of bio-molecules in plants which can act as capping/stabilizing and reducing agents and so increases the rate of reduction and stabilization of synthesized nanoparticles. Among all organisms plants seem to be the best candidates for biosynthesis of silver nanoparticles and they are suitable for large scale biosynthesis. Nanoparticles produced by plants are more stable and the rate of synthesis is faster than in the case of microorganisms. This review focuses on the green synthesis of silver nanoparticles using various plant sources.

Plant Extract name	**Size of Nanoparticles (nm)**	**Antibacterial /Antifungal against**	**Title**	**Author and year**
1.*Annona reticulata* unripe fruit extract	$AgNO_3$ 12.72nm	-	Biosynthesis of silver nanoparticles using unripe fruit extract of *Annona Reticulata l* and its characterization.	Ghotekar K.S. *et al*.,(2015)
2.*Benincasa hispida* seed extract	$HAuCl_4$ 5 – 40nm	*Fusarium oxysporium, Trichoderma viridae*	Biosynthesis of elixir of life.	Singh *et al.*, (2013)
3.Banana Peel extract	$AgNO_3$ 23.7nm	-	Green synthesis and characterization of silver nanoparticles using banana peels and their antimicrobial activity against representative micro-organism.	Haytham M.M. Ibrahim (2015)
4.*Citrus sinensis* peel	$AgNO_3$ 10 – 35nm	*E.coli, Pseudomonas aeruginosa, Staphylococcus aureus.*	Biosynthesis of silver nanoparticles using *citrus sinensis* peel extract and its antibacterial activity.	S.kaviya *et al.*, (2011)
5.*Carica papaya* leaf extract	$AgNO_3$ 25 – 35nm	_	Investigation of biogenic silver nanoparticles green synthesized from *Carica papaya.*	Konjari *et al.*, (2015)

6.*Cucurbita lagenaria* fruit extract	$AgNO_3$ 11.2 – 50.12nm	*Klebsiella pneumoniae, NCIM 2719 and Clostridium acetobutilicum.*	Rapid biosynthesis of silver nanoparticles using bottle gourd fruit extract and potential application as bactericide.	Abasaheb *et al*., (2013)
7.*Elaeocarpus ganitrus* extract	$AgNO_3$ -	*Staphylococcus aureus.*	Novel scientific appraisal of *Elaeocarpus ganitrus* the rudraksha: Nanosilver synthesis with aspects of variation in concentration antimicrobial activity and in-vitro biocompatibility	Dwivedi P.*et al.*, (2014)
8.*Elettaria cardamomum* seed extract	$HAuCl_4$ 432.3nm	_	Green synthesis of gold nanoparticles using *Elettaria cardamomum* (Elaichi) aqueous extract.	Pattanayak and Nayak, (2013)
9.*Fenugreek* seed extract	$AgNO_3$ 174nm	_	Green synthesis and characterization of silver nanoparticles using *fenugreek* seed extract.	Angelina R..D.E *et.al.* (2013)
10.Flaxseed Hydroalcoholic extract	$AgNO_3$ 9.22nm	*E,coli, Staphylococcus aureus* and *mycotoxin producing fungus*	Synthesis of silver nanoparticles using flaxseed hydroalcoholic extract and its antimicrobial activity.	Sharbidre A.A. and Kasote M.D. (2013)

11.*Foeniculum vulgare* seed extract	$AgNO_3$ 11- 25nm	*Pseudomonas aeruginosa, Proteus mirabilis,Escherichia coli, shigella flexnari* and *klebsiella pneumoniae.*	Rapid green synthesis of silver nanoparticles using seed extract of *Foeniculum vulgare* and screening of its antibacterial activity.	Showmya *et al.*, (2012)
12.Jackfruit peel extract	–	–	Agricultural wastes of jackfruit peel nano-porous adsorbent for removal of rhodamine dye.	M.Jayarajan *et al*., (2011)
13.*Lycopersicon esculentum* extract	$AgNO_3$ 50nm	*Escherichia coli*	Antimicrobial activities of silver nanoparticles synthesized from *lycopersicon esculentum* extract.	M.Swarnali *et al.*, (2014)
14.Lemon peels extract	$AgNO_3$ -	Dermatophytes	Lemon peels mediated synthesis of silver nanoparticles and its antidermatophytic activity.	N.Najimo S *et al.*, (2014)
15.Mango peel extract	$AgNO_3$ 7-27nm	*Escherichia coli, S.aureus*	Mango peel extract mediated novel route for synthesis of silver nanoparticles and antibacterial application of silver nanoparticles loaded onto non-woven fabric.	Ning yang, Wei-hong (2013)
16.*Mangifera indica* extract	$AgNO_3$ 100nm	*Escherichia coli* and *Pseudomonas aeruginosaby*	Synthesis and characterization of silver nanoparticles from *Mangifera indica* and its antibacterial activity.	Dhanapal *et al.* (2015)

17.*Musa paradisiacal* peel extract	$AgNO_3$ 20nm	Fungal as well as bacterial	Banana peel extract mediated novel route for the synthesis of silver nanoparticles	A.Bankar *et al.* (2010)
18.*Nerium oleander* extract	$AgNO_3$ -	*Escherichia coli* and *Bacillus subtilis*	Biological synthesis of silver nanoparticles from *Nerium oleander* and its antibacterial and antioxidant property.	R.Subbaiya *et al*. (2014)
19.Olive leaf extract	$AgNO_3$ 20-25nm	*S.aureus, Pseudomonas aeruginosa, Escherichia coli*	Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity.	K.H.M.Mostafa *et al*. (2014)
20.*Solanum tricobatum, Syzygium cumini, Centella asiatica* and *Citrus sinensis* Extract	$AgNO_3$ 52nm 53nm 42nm 41nm	*Staphylococcus aureus, Pseudomonas aeruginosa,, Escherichia coli, Klebsiella*	Ecofriendly Synthesis of silver nanoparticles from commercially available plant powder and their antibacterial property.	Logeswari.*et.al.* (2013),
21.Pomegranate peels extract	$AgNO_3$ 5±1.5nm	-	Biosynthesis of silver nanoparticles from biowaste pomegranate peels	Naheed Ahmad, Seema Sharma (2012)
22.*Sesamum indicum* seed extract	$AgNO_3$ 13nm	*Escherichia coli*	The Antibacterial activity of silver nanoparticles produced in the plant *Sesamum indicum* seed extract: A Green method against multi drug resistance *Escherichia coli*	Bokaeian *et al.* (2014)

23.*Syzygium cumini* seed extract	$AgNO_3$ -	-	Biosynthesis of silver nanoparticles from *syzygium cumini* seed extract and evaluation of their in vitro antioxidant activities.	Banerjee *et al.* (2011)
24.*Vitis Vinifera* leaves and seeds extract	$HAuCl_4$ 13.1and 8.9nm	-	Biosynthesis of gold nanoparticles using extract of grape(*vitis vinifera*)leaves and seeds.	Ismail *et al.* (2014)
25.*Tangerine* peel extract	$AgNO_3$ 2:1)30.29±5.1 nm 1:1)16.68±5.7 nm 1:2)25.85±8.4 nm	-	Ecofriendly production of silver nanoparticles from peel of tangerine for degradation of dye.	Alzahrani *et al.* (2015)

No research papers were found on the antibacterial effect along with antibiotic revival using nanoparticles synthesized from *Litchi chinensis*, *Mangifera indica, Juglans regia* peels extract on the bacteria isolated from urine sample of infected patient suffering from urinary tract infection. So far there are research paper based on the potency of nanoparticles extracted against a bacteria which is purchased from a source, therefore, we choose to carry out our further research work on silver nanoparticles synthesized from the peels extract of litchi, mango and walnut and will examine their efficiency on the control of bacteria *E.coli* causing urinary tract infection.

This method of synthesizing nanoparticles from waste fruit peels is quite cost efficient, organic and eco-friendly as compared to other chemical and physical method, simultaneously helping in waste management and bringing out the best from the waste as well.

OBJECTIVES

- Collection of the clinical sample from different sources. (S.N. Medical College Agra, pathology laboratories of Agra).
- Isolation and identification of the bacteria from the sample collected.
- Collection of waste fruit peels *(Litchi chinensis, Mangifera indica and Juglans regia)* preparation of powder of the peels collected.
- Biogenic synthesis of silver nanoparticles from the waste of the fruit peels.
- Screening of antibacterial effect of silver nanoparticles synthesized.
- Characterization of silver nanoparticles (UV-Vis, XRD, FTIR and SEM).

Methodology

1. Isolation and Purification of microbes:

Collection of the pathogenic bacteria (*Escherichia coli)* causing urinary tract infection from pathological laboratory and hospitals of Agra. We will culture the collected bacteria using selective and differential medium. Sterilization of glassware is important before use to avoid contamination. Sterilization of Petri dishes by incubating them at 27-28degree in the oven for 2-3 days. The growth of microbial colony will appear on the plates after 24-72 hours, transferring of bacteria to other Petri dish using loop and further streaking for purification. The process of Sub culture will be in practice until pure culture obtained.

2. Collection of waste peel of fruits and powder preparation:-

The peel of fruits *Litchi chinensis, Mangifera indica* and *Juglan regia* will be collected from the local market of Agra. Then after washing of the peels using distilled water and shade drying will be carry out, when the peels will be well dry, the next step will be of grinding using domestic grinder, properly washed and dried to avoid contamination. Powder obtained will be stored in an airtight container for the future use.

3. Biosynthesis of Silver Nanoparticles:

We will prepare three different concentrations of silver nitrate ($AgNO_3$) 1mM, 2mM and 3mM in distilled water and then will add fruit peel powder solution to each concentration of silver nitrate for the reduction of Ag+ ions into Ag and will observe them at different time intervals of 24 hours for the production of AgNPs. (Banerjee *et al*. 2011 and Khadri *et al*. 2013).
The mechanism of production of AgNPs from the plant peel extract will be explained by the following reaction:

$$Ag{+}NO_3 + \text{PLANT PEEL EXTRACT} \rightarrow Ag^0\text{NPs} + \text{byproducts}$$

4. Characterization of silver nanoparticles:

The silver nanoparticles synthesized will be characterize with the help of **UV-Vis** spectrophotometer, Scanning electron microscopy **(SEM)**, X-Ray diffraction **(XRD)** and Fourier Transform Infra Red **(FTIR).** With the help of uv-vis spectrophotometer, we will monitor the reduction of pure silver ions by measuring the uv-vis spectrum of the sample prepared. (Shanker *et al*.2003). now further analysis of structure and composition by XRD will done and to know the bioreduction of Ag+ ions to silver nanoparticles which is due to the reduction by capping material of plant extract FTIR analysis will be done.SEM will be performed to confirm the size of the silver nanoparticles. (K. Mallikarjuna *et al*. 2014).

5. Antibacterial Screening of Silver Nanoparticles:

The silver nanoparticles synthesized will be examined for their antibacterial activity on the selected pathogenic microbe *Escherichia coli* and on samples contaminated with *Escherichia coli* from different sources. Antibacterial assay will be examined by measuring the minimum zone of inhibition by disc diffusion method and agar well diffusion method (Vincent J. *et al*., 1944 and Perez C. *et al*.1990.), taking pure plant peel extract and pure $AgNO_3$ solution as control. The zone that will be obtained by the above methods will prove the antibacterial activity of silver nanoparticles synthesized from the peels of fruit and the one giving the maximum zone of inhibition out of the three fruit peel will be selected for the further research. (Driscol J.A *et al*. 2012)

6. Screening of the combination of antibiotics with biosynthesized Silver Nanoparticles on pathogenic microbe:

Biosynthesized Silver nanoparticles will be mixed with an antibiotic which is showing resistance to *E.coli* and this combination will be observed for the antibacterial property against *Escherichia coli*, a very low dose of antibiotic will be combined with AgNPs. Diameter of Zone of inhibition will determine the efficiency of the combination used.

Significance of the research

Nanotechnology is of great importance in today's world, development is the ongoing process and nanotechnology is adding on to this developmental process. Silver nanoparticles synthesized using nanotechnology is proving beneficial due to its vast characteristics in various fields, making it most widely used. One of its most beneficial characteristic is the antibacterial activity it shows against the various pathogenic microbes. This property of silver nanoparticles is helpful in solving the global problem of multi drug resistance.

The outcome of this research will be focus on the use of waste fruits peel for the synthesis of silver nanoparticles, to treat infectious disease of urinary tract infection caused by bacteria *Escherichia coli*. Antibiotics help in the treatment of many diseases but over use of them are creating health hazardous conditions in human beings as they are developing antibiotic resistance in them. Thus treatments of severe diseases are becoming difficult as the body of the patient did not respond to the antibiotic treatment given. Therefore, to control the misuse of antibiotic, silver nanoparticles will be brought forward for treatment of various infectious diseases. One such disease is urinary tract infection. Silver nitrate is highly effective on the activity of microbes. Silver-based compounds are highly toxic to microorganisms. Thus, in the present study an attempt will be made to produce nano drug synthesized from novel waste fruits peel against urinary tract infection.

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